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(54) Title: USE OF A PROTEIN KINASE C INHIBITOR TO ENHANCE THE CLINICAL EFFICACY OF ANTI-NEOPLASTIC CHEMOTHERAPEUTIC AGENTS AND RADIATION THERAPY

(57) Abstract: Methods are disclosed for treating a neoplasm which comprises administering to a mammal in need thereof, an anti-neoplastic agentor therapeutic radiation in combination with 3-[1-(1-(pyridin-2-ylmethyl)piperidin-4-yl)-indol-3-yl]-4-(1-methylindol-3-yl)-1H-pyrrole-2,5-dione or a pharmaceutically acceptable salt or solvate thereof, and for enhancing the anti-neoplastic effect of anti-neoplasticagents or therapeutic radiation which comprises administering to a mammal in need thereof, 3-[1-(1-(pyridin-2-ylmethyl)piperidin-4-yl)-indol-3-yl]-4-(1-methylindol-3-yl)-1H-pyrrole-2,5-dione in combination with said anti-neoplastic agent or said therapeuticradiation having an anti-neoplastic effect.

USE OF A PROTEIN KINASE C INHIBITOR TO ENHANCE THE CLINICAL EFFICACY OF ANTI-NEOPLASTIC CHEMOTHERAPEUTIC AGENTS AND RADIATION THERAPY

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention is broadly directed to a method for enhancing the anti-neoplastic effects of anti-neoplastic chemotherapies and radiation therapies with a protein kinase C (PKC) inhibitor. The present invention is particularly directed to the use of an isozyme selective PKC inhibitor in combination with an anti-neoplastic agent or therapeutic radiation to enhance their anti-neoplasm effects in treatment of neoplasms.

2. Description of Related Art

Therapeutic treatments have been developed over the years to treat neoplasms. There are two major non-surgical approaches to treat neoplasms: 1) chemotherapy employing anti-neoplastic agents, and 2) radiation therapy. Other approaches include immunotherapies and antiangiogenic therapies. Anti-neoplastic agents and radiation cause cytotoxic effects, preferentially to tumor cells, and cause cell death.

Studies have shown that therapeutic radiation and most groups of anti-neoplastic agents assert their cytotoxic effects by activating programmed cell death or apoptosis. A balance between the activities of apoptotic and antiapoptotic intracellular signal transduction pathways is important in determining whether a cell will undergo apoptosis in response to the above-mentioned chemotherapy as well as radiation therapy.

PKC inhibitors have been proposed for cancer therapy (see, for example, U.S. 5,552,391) and it has been indicated that PKC activities exert antiapoptotic effects, especially in response to anti-neoplastic agents or radiation therapies. In particular, studies have shown that activation of PKC inhibits apoptosis induced by anti-neoplasm agents such as 1-β-D- arabinofuranosylcytosine or Ara-c, etoposide or VP-16, *cis*-diamminedichloroplatinum (II) or *cis*-platin, doxorubicin or adriamycin, 2- chloro-2-deoxyadenosine, 9-β-D-arabinosyl-2-fluoroadenine, and glucocorticoids, and gamma

irradiation therapy. There also have been indications that down regulation of PKC activities in tumor cells enhances apoptosis stimulated by anti-neoplastic agents. PKC activation has been shown to attenuate gamma-irradiation induced cell death (Blumberg PM, Acs P, Bhattacharyya DK, Lorenzo PS. Inhibitors of Protein Kinase C and related receptors for the lipophilic second-messenger sn-1,2-diacylglycerol. IN: J. S. Gutkind (ed.), Signaling Networks and Cell Cycle Control. Humana Press: Totowa, NJ, 2000; pps. 347-364).

Anticancer therapies, especially chemotherapies and radiation therapy , while frequently used, often produce some response in the malignant disease but are rarely curative. The importance of normal cells and tissues to support the growth of tumors have long been recognized. The observations of Van der Kolk (Pluda JM. Tumor-Associated Angiogenesis: Mechanisms, Clinical Implications, and Therapeutic Strategies. Seminars in Oncology **24**:203-218 (1997) ("Pluda"), Jones (Norrby K. Angiogenesis: new aspects relating to its initiation and control. APMIS **105**:417-437 (1997) ("Norrby") and Paget (Fox SB, Harris AL. Markers of tumor angiogenesis: clinical applications in prognosis and anti-angiogenic therapy. Investigational New Drugs **15**:15-28 (1997) ("Fox 1")) more than 100 years ago documented this knowledge in the clinical science literature. Fifty years ago, Algire and Chalkey (Risau W. What, if anything, is an angiogenic factor? Cancer and Metastasis Reviews **15**:149-151 (1996) ("Risau")) reported that host vascular reactions could be elicited by growing tumors and described in detail the extent and tumor-specific nature of the induction of host capillaries by transplanted tumors. The central hypothesis of Algire and Chalkey was that vascular induction by solid tumors may be the major, and possibly, the only distinguishing factor leading to tumor growth beyond normal tissue control levels. By the late 1960s, Folkman and his colleagues (Singh RK, Fidler IJ. Regulation of Tumor Angiogenesis by Organ-Specific Cytokines. Current Topics in Microbiology & Immunology **213**:1-11 (1996) ("Singh") ; Iruela-Arispe ML, Dvorak HF. Angiogenesis: a dynamic balance of stimulators and inhibitors. Thrombosis and Haemostasis **78**: 672-677 (1997) ("Iruela-Arispe"); Nguyen M. Angiogenic factors as tumor markers. Investigational New Drugs **15**: 29-37 (1997) ("Nguyen")) had begun the search for a tumor angiogenesis factor (TAF) and in 1971, Folkman proposed "antiangiogenesis" as a means of holding tumors in a non-vascularized dormant state (Toi

M, Taniguchi T, Yamamoto Y, et al. Clinical Significance of the Determination of Angiogenic Factors. European Journal of Cancer 32A:2513-2519 (1996) ("Toi")). Vascular endothelial growth factor (VEGF) is now recognized as a major angiogenesis factor in tumors. The intracellular signaling pathway of this endothelial cell specific growth factor involves the enzyme protein kinase Cβ (PKCβ), therefore potent selective inhibitors of PKCβ will block the mitogenic and migratory effects of VEGF on endothelial cells. Basic fibroblast growth factor and likely other angiogenic factors utilize PKCβ in their intracellular signal transduction pathways. Small molecule inhibitors of PKCβ will, therefore in addition to enhancing malignant cell killing by chemotherapeutic agents and radiation therapies, be potent inhibitors of tumor angiogenesis and be widely applicable agents for the treatment of solid tumors.

Most solid tumors increase in mass through the proliferation of malignant cells and stromal cells including endothelial cells leading to formation of a tumor vasculature (Pluda). Since active angiogenesis is a critical component of the mass expansion of most solid tumors, this process is a valid target for therapy (Norrby). Angiogenesis, vasculature formation, during malignant growth is a complex process. Elucidation of the process has involved recognition of angiogenic stimuli such as hypoxia and nutrient deprivation, recognition of angiogenic factors produced by malignant cells, fibroblasts and tumor infiltrating leukocytes and recognition that there may be a concomitant decrease in negative angiogenic regulators by the same three cell populations within the tumor for angiogenesis to occur (Norrby; Fox 1, Risau; Singh; Iruela-Arispe). Endogenous stimulators of angiogenesis, that is angiogenic factors, include vascular endothelial growth factor (VEGF), transforming growth factors α and β (TGFα and TGFβ), acidic and basic fibroblast growth factor (a FGF and bFGF), epidermal growth factor (EGF), platelet-derived endothelial cell growth factor/thymidine phosphorylase (PD-ECGF/TP), angiogenin, pleiotropin, hepatocyte growth factor (HGF), interleukins-1,-6,-8 (IL-1, IL-6, IL-8), placental growth factor, E-selectin, tumor necrosis factor-α (TNF-α), heparinase, angiopoeitin, and hypoxia-inducible factor (Singh; Iruela-Arispe; Nguyen; Toi; Takebayashi Y, Akiyama S, Akiba S, et al. Clinicopathologic and Prognostic Significance of an Angiogenic Factor, Thymidine Phosphorylase, in Human Colorectal Carcinoma. J Natl Cancer Inst 88:1110-71 (1996) ("Takebayashi 1"); Takebayashi Y,

Yamada K, Miyadera K, et al. The Activity and Expression of Thymidine Phosphorylase in Human Solid Tumours. European Journal of Cancer **32A**:1227-1232 (1996) ("Takebayashi 2"); O'Brien TS, Fox SB, Dickinson AJ, et al. Expression of the Angiogenic Factor Thymidine Phosphorylase/Platelet-derived Endothelial Cell Growth Factor in Primary Bladder Cancers. Cancer Research **56**:4799-4804 (1996) ("O'Brien"); Fox SB, Westwood M, Moghaddam A, et al. The angiogenic factor platelet-derived endothelial cell growth factor/thymidine phosphorylase is up-regulated in breast cancer epithelium and endothelium. British Journal of Cancer **73**:275-280 (1996) ("Fox 2"); Griffiths L, Dachs G, Bicknell R, et al. The Influence of Oxygen Tension and pH on the Expression of Platelet-derived Endothelial Cell Growth Factor/Thymidine Phosphorylase in Human Breast Tumor Cells Grown *in Vitro* and *in Vivo*. Cancer Research **57**:570-572 (1997) ("Griffiths"); Yoshiji H, Harris S, Thorgeirsson U. Vascular Endothelial Growth Factor Is Essential for Initial but not Continued *in Vivo* Growth of Human Breast Carcinoma Cells. Cancer Research **57**:3924-3928 (1997) ("Yoshiji"); Abedi H, Zachary I. Vascular Endothelial Growth Factor Stimulates Tyrosine Phosphorylation and Recruitment to New Focal Adhesions of Focal Adhesion Kinase and Paxillin in Endothelial Cells. The Journal of Biological Chemistry **272**:15442-15451 (1997) ("Abedi")). Endogenous negative angiogenic factors include vascular endothelial growth factor (VEGF), transforming growth factors α and β (TGFα and TGFβ), acidic and basic fibroblast growth factor (a FGF and bFGF), epidermal growth factor (EGF), platelet-derived endothelial cell growth factor/thymidine phosphorylase (PD-ECGF/TP), angiogenin, pleiotropin, hepatocyte growth factor (HGF), interleukins-1,-6,-8 (IL-1, IL-6, IL-8), placental growth factor, E-selectin, tumor necrosis factor-α (TNF-α), heparinase, angiopoeitin, and hypoxia-inducible factor (id.). Endogenous negative regulators or inhibitors of angiogenesis include thrombospondin, platelet factor IV, interferon-α, interferon-β, protamine, cartilage-derived inhibitors, angiostatin, endostatin, plasminogen activator inhibitor (PAI) and the tissue inhibitor of metalloproteinases (TIMPs) (Pluda; Norrby; Singh; Abedi) Preclinical and clinical studies have shown that malignant cells in culture and tumors *in vivo* can and, most often, do express an array of angiogenesis stimulators and negative regulators. Clearly, angiogenesis is a highly complex and closely regulated process and it is not surprising that vasculature in malignant masses is often

poorly formed, irregular, lacking complete structure and inadequate to feed the tissue (Anand-Apte B, Bao L, Smith R, et al. A review of tissue inhibitor of metalloproteinases-3 (TIMP-3) and experimental analysis of its effect on primary tumor growth. Biochem. Cell Biol. **74**: 853-862 (1996); Fukumura D, Yuan F, Monsky WL, et al. Effect of host microenvironment on the micro-circulation of human colon adenocarcinoma. American Journal of Pathology **151**: 679-688 (1997); Less JR, Posner MC, Skalak TC, et al. Geometric resistance and microvascular network architecture of human colorectal carcinoma. Microcirculation **4**: 25-33 (1997)). The combination of antiangiogenic agents with standard therapies appears to be synergistic (Jain RK. The Eugene M. Landis Award Lecture 1996. Delivery of molecular and cellular medicine to solid tumors. Microcirculation **4**: 1-23 (1997)).

The most clear-cut, direct-acting, most frequently found angiogenic factor in cancer patients is vascular endothelial growth factor (VEGF) (Pluda; Norrby; Fox 2; Risau; Jain RK. The Eugene M. Landis Award Lecture 1996. Delivery of molecular and cellular medicine to solid tumors. Microcirculation **4**: 1-23 (1997)). VEGF expression has been positively associated with primary breast cancer, brain tumors, cervical neoplasias, lung cancer, stomach, colon cancer and others. Furthermore, the up-regulation of the VEGF receptors, flt-1 and KDR, has been observed in tumor-associated endothelial cells in a variety of tumors including breast cancer, brain tumors, kidney tumors, bladder cancer, ovarian cancer and colon cancer. The signal transduction pathways of the KDR/Flk-1 and Flt-1 receptors include tyrosine phosphorylation, activation of PLCγ, diacylglycerol generation, and PI-3 kinase with downstream activation of protein kinase C (PKC) and activation of the MAP kinase pathway (Teicher BA , ed. Antiangiogenic Agents in Cancer Therapy. New Jersey: The Humana Press, Inc., 1999, Vol. 4; Xia P, Aiello LP, Ishii H, Jiang ZY, Park DJ, Robinson GS, Takagi H, Newsome WP, Jirousek MR, and King GL, Characterization of vascular endothelial growth factor's effect on the activation of protein kinase C, its isoforms, and endothelial cell growth, Journal of Clinical Investigation **98**: 2018-2026 (1996); Guo D, Jia Q, Song HY, Warren RS, and Donner DB, Vascular endothelial cell growth factor promotes tyrosine phosphorylation of mediators of signal transduction that contain SH2 domains. Association with endothelial cell proliferation, Journal of Biological Chemistry **270**: 6729-6733 (1995);

Sawano A, Takahashi T, Yamaguchi S and Shibuya M, The phosphorylated 1169-tyrosine containing region of flt-1 kinase (VEGFR-1) is a major binding site for PLCgamma, Biochemical & Biophysical Research Communications 238: 487-491 (1997)). This intracellular signal transduction pathway may be common, at least in part, for most angiogenic factors.

Protein kinase C is a gene family consisting of at least 12 isoforms (Mohammadi M, Dikic I, Sorokin A, Burgess WH, Jaye M, and Schlessinger J, Identification of six novel autophosphorylation sites on fibroblast growth factor receptor 1 and elucidation of their importance in receptor activation and signal transduction, Molecular & Cellular Biology **16**, 977-989 (1996)). Based on differing substrate specificity, activator requirements and subcellular compartmentalization, it is hypothesized that activation of individual protein kinase C isoforms preferentially elicits specific cellular responses (id.).

There is a need in the art to develop therapeutic agents which enhance the apoptotic signal transduction pathways in malignant cells and inhibit the signal transduction pathways of angiogenic factors in tumors, and thereby enhance the clinical efficacy of anti-neoplastic agents and radiation therapy.

SUMMARY OF INVENTION

It is an object of the invention to provide methods for treating a neoplasm.

It is another object of the invention to provide methods for enhancing the anti-neoplastic effect of an anti-neoplastic agent.

It is still another object of the invention to provide methods for enhancing the anti-neoplastic effect of therapeutic radiation.

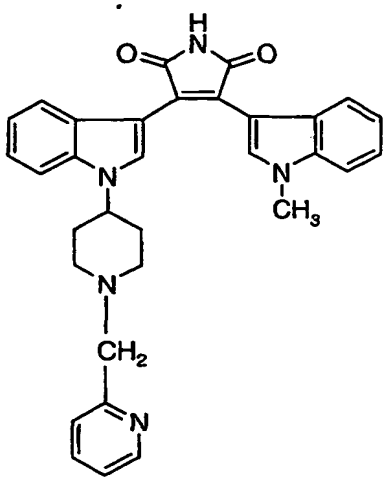
It is still another object of the invention to provide for the manufacture of medicaments and products for the treatment of neoplasms, and for enhancing the anti-neoplastic effects of anti-neoplastic agents and therapeutic radiation.

These and other objects of the invention are provided by one or more of the embodiments described below.

In one embodiment of the invention there is provided a method for treating a neoplasm which comprises administering to a mammal in need thereof, an anti-neoplastic agent or therapeutic radiation in combination with 3-[1-(1-(pyridin-2-

ylmethyl)piperidin-4-yl)-indol-3-yl]-4-(1-methylindol-3-yl)-1H-pyrrole-2,5-dione (which is the compound of Formula I)

or a pharmaceutically acceptable salt or solvate thereof.



Formula I

In another embodiment of the invention there is provided a method for enhancing the anti-neoplastic effect of an anti-neoplastic agent and therapeutic radiation which comprises administering to a mammal in need thereof, a compound of Formula I in combination with said anti-neoplastic agent or therapeutic radiation having an anti-neoplastic effect.

In another embodiment of the invention there are provided uses for the compound of Formula I, or pharmaceutically acceptable salts or solvates thereof, for the manufacture of medicaments and products for treatment of neoplasms, and enhancing the treatment of neoplasms by anti-neoplastic agents and radiation therapy.

The present invention provides the art with a method for increasing apoptotic effects in malignant cells and inhibiting the signal transduction of angiogenic factors in tumors, and is thus effective in enhancing the anti-neoplasm effects of anti-neoplastic chemotherapies and radiation therapies.

DETAILED DESCRIPTION OF THE INVENTION

It is a discovery of the present invention that use of a compound of Formula I, reduces or inhibits anti-apoptotic effects in a malignant cell and inhibits the intracellular signal transduction of angiogenic factors. Consequently, this compound can be used to enhance the anti-neoplasm effects of anti-neoplastic agents and radiation therapies.

This compound may be prepared by methods known in the art (see, for example, U.S. Patent No. 5,668,152) In accordance with the present invention, this compound is administered in combination with other anti-neoplasm therapies to a mammal in need of such treatment. In particular, this compound can be used to enhance the anti-neoplasm effects of chemotherapies and radiation therapies.

Because it contains a basic moiety, the compound of Formula I can also exist as pharmaceutically acceptable acid addition salts. Acids commonly employed to form such salts include inorganic acids such as hydrochloric, hydrobromic, hydroiodic, sulfuric and phosphoric acid, as well as organic acids such as para-toluenesulfonic, methanesulfonic, oxalic, para-bromophenylsulfonic, carbonic, succinic, citric, benzoic, acetic acid, and related inorganic and organic acids. Such pharmaceutically acceptable salts thus include sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, 2-butyne-1,4-dioate, 3-hexyne-2, 5-dioate, benzoate, chlorobenzoate, hydroxybenzoate, methoxybenzoate, phthalate, xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, hippurate, β- hydroxybutyrate, glycolate, maleate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate and the like. Particularly the hydrochloric and mesylate salts are used.

In addition to pharmaceutically acceptable salts, other salts also can exist. They may serve as intermediates in the purification of the compound of Formula I, in the preparation of other salts, or in the identification and characterization of the compound or intermediates.

The pharmaceutically acceptable salts of the compound of Formula I can also exist as various solvates, such as with water, methanol, ethanol, dimethylformamide, ethyl acetate and the like. Mixtures of such solvates can also be prepared. The source of such solvate can be from the solvent of crystallization, inherent in the solvent of preparation or crystallization, or adventitious to such solvent.

The compound of Formula I is used in combination with conventional anti-neoplasm therapies to treat mammals, especially humans, with neoplasia. The procedures for conventional anti-neoplasm therapies, including chemotherapies using anti-neoplastic agents and therapeutic radiation, are readily available, and routinely practiced in the art, *e.g.*, see Harrison's PRINCIPLES OF INTERNAL MEDICINE 11th edition, McGraw-Hill Book Company.

Neoplasia is characterized by abnormal growth of cells which often results in the invasion of normal tissues, *e.g.*, primary tumors or the spread to distant organs, *e.g.*, metastasis. The treatment of any neoplasia by conventional non-surgical anti-neoplasm therapies can be enhanced by the present invention. Such neoplastic growth includes but not limited to primary tumors, primary tumors that are incompletely removed by surgical techniques, primary tumors which have been adequately treated but which are at high risk to develop a metastatic disease subsequently, and an established metastatic disease.

Specifically, the PKC inhibitor of Formula I above can enhance the anti-neoplasm effects of an anti-neoplastic agent. The wide variety of available anti-neoplastic agents are contemplated for combination therapy in accordance with present invention. In a preferred embodiment, anti-neoplastic agents that assert their cytotoxic effects by activating programmed cell death or apoptosis are used in combination with the described PKC inhibitor. The anti-neoplastic agents contemplated in accordance with the present invention include, but are not limited to alkylating agents, including busulfan, chlorambucil, cyclophosphamide, iphosphamide, melphalan, nitrogen mustard, streptozocin, thiotepa, uracil nitrogen mustard, triethylenemelamine, temozolomide, and SARCnu; antibiotics and plant alkaloids including actinomycin-D, bleomycin, cryptophycins, daunorubicin, doxorubicin, idarubicin, irinotecan, L-asparaginase, mitomycin-C, mitramycin, navelbine, paclitaxel, docetaxel, topotecan, vinblastine, vincristine, VM-26, and VP-16-213; hormones and steroids including 5α-reductase

inhibitor, aminoglutethimide, anastrozole, bicalutamide, chlorotrianisene, DES, dromostanolone, estramustine, ethinyl estradiol, flutamide, fluoxymesterone, goserelin, hydroxyprogesterone, letrozole, leuprolide, medroxyprogesterone acetate, megestrol acetate, methyl prednisolone, methyltestosterone, mitotane, nilutamide, prednisolone, SERM3, tamoxifen, testolactone, testosterone, triamicnolone, and zoladex; synthetics including all-trans retinoic acid, BCNU (carmustine),CBDCA carboplatin (paraplatin), CCNU (lomustine), cis-diaminedichloroplatinum (cisplatin), dacarbazine, gliadel, hexamethylmelamine, hydroxyurea, levamisole, mitoxantrone, o, p'-DDD (lysodren, mitotane), oxaliplatin, porfimer sodium, procarbazine, GleeVec; antimetabolites including chlorodeoxyadenosine, cytosine arabinoside, 2'-deoxycoformycin, fludarabine phosphate, 5-fluorouracil, 5-FUDR, gemcitabine, 6-mercaptopurine, methotrexate, MTA, and thioguanine; and biologics including alpha interferon, BCG, G-CSF, GM-CSF, interleukin-2, herceptin; and the like.

In a preferred embodiment of the invention, the anti-neoplastic agent is selected from the group consisting of alkylating agents, antibiotics and plant alkaloids, hormones and steroids, synthetic agents having anti-neoplastic activity, antimetabolites and biological molecules having anti-neoplastic activity.

In a preferred embodiment of the invention the antineoplastic agent is selected from the group consisting of Ara-c, VP-16, *cis*-platin, adriamycin, 2-chloro-2-deoxyadenosine, 9-β-D-arabinosyl-2-fluoroadenine, carboplatin, gemcitabine, paclitaxel, BCNU, 5-fluorouracil, and doxorubicin.

All the neoplastic conditions treatable with such anti-neoplastic agents can be treated in accordance with the present invention by using a combination of the compound of Formula I with one or more anti-neoplastic agents. The anti-neoplastic agents assert their cytotoxicity or anti-neoplasm effects in a variety of specific neoplastic conditions. For example, Ara-c is normally used for treatment of childhood-null acute lymphoid leukemia (ALL), thymic ALL, B-cell ALL, acute myeloid leukemia, acute granulocytic leukemia and its variants, non-Hodgkins lymphoma, myelomonocytoid leukemia, acute megakaryocytoid leukemia and Burkitt's lymphoma, Adult-B-ALL, acute myeloid leukemia, chronic lymphoid leukemia, chronic myeloid leukemia, and T cell leukemia. VP-16 is normally used for treatment of testicular carcinoma, small and

large non-small cell lung carcinoma, Hodgkin's lymphoma, non-Hodgkin's lymphoma, choriocarcinoma, Ewing's sarcoma, and acute granulocytic leukemia. *Cis*-platin can be employed for treatment of testicular carcinoma, germ cell tumors, ovarian carcinomas, prostate cancer, lung cancer, sarcomas, cervical cancer, endometrial cancer, gastric cancer, breast cancer, and cancer of the head and neck. 2-Chloro-2-deoxyadenosine and 9-β-D-arabinosyl-2-fluoroadenine can be used to treat chronic lymphoid leukemia, lymphomas and hairy cell leukemia. Doxorubicin can be used to treat acute granulocytic leukemia and its variants, ALL, breast cancer, bladder cancer, ovarian cancer, thyroid cancer, lung cancer, Hodgkin's lymphoma, non-Hodgkin's lymphoma, sarcomas, gastric carcinoma, prostate cancer, endometrial cancer, Wilm's tumor and neuroblastoma. Clinical effects of anti-neoplastic agents in all neoplastic conditions treatable with anti-neoplastic agents including the ones discussed above can be potentiated by use of a combination therapy with the identified PKC inhibitor in accordance with the present invention.

The PKC inhibitor identified in the present invention can also enhance the anti-neoplasm effects of radiation therapy. Usually, radiation is used to treat the site of a solid tumor directly or administered by brachytherapy implants. The various types of therapeutic radiation which are contemplated for combination therapy in accordance with the present invention are those used in the treatment of cancer which include, but are not limited to X-rays, gamma radiation, high energy electrons and High LET (Linear Energy Transfer) radiation such as protons, neutrons, and alpha particles. The ionizing radiation is employed by techniques well known to those skilled in the art. For example, X-rays and gamma rays are applied by external and/or interstitial means from linear accelerators or radioactive sources. High-energy electrons can be produced by linear accelerators. High LET radiation is also applied from radioactive sources implanted interstitially.

The following examples are provided merely to further illustrate the present invention. The scope of the present invention is not to be construed as merely consisting of the following examples. In each of the following examples, the compound of Formula I is administered as the dihydrochloride salt, and the amounts administered are given in terms amounts of the dihydrochoride salt.

The murine Lewis lung carcinoma or human T98G glioblastoma mutliforme, human SW2 small cell lung carcinoma, human Calu-6 non-small cell lung carcinoma, human SKOV-3 ovarian carcinoma, human HT-29 colon carcinoma, HS746T gastric carcinoma, Hep3B hepatocellular carcinoma, CaKi1 renal cell carcinoma or human MX-1 breast carcinoma was implanted in male C57Bl mice or male or female nude mice and the tumor-bearing animals were treated with the compound alone or along with anticancer chemotherapy (paclitaxel, carboplatin, gemcitabine, BCNU, 5-fluorouracil or cisplatin) or with locally administered fractionated radiation therapy. Specifically, for most experiments, tumor cells prepared from a brie of donor tumors (5 x 10^6 cells) were implanted subcutaneously in a hind-leg of mice (Charles River). Intracranial tumors were implanted by injecting 10^4 T98G cells in 1.5 microliters. Intraperitoneal tumors were implanted by injecting 5 x 10^6 SKOV-3 tumor cells intraperitoneally into female nude mice. Treatment with the compound (3, 10 or 30 mg/kg) administered orally twice per day for schedules lasting 2 weeks or more. Each standard chemotherapeutic agent was administered in a full murine dose regimen for that agent. Each treatment group as well as a group of untreated control animals consisted of 5 animals per group in each experiment. Subcutaneous tumor response was monitored by tumor volume measurement performed twice per week over the course of 60-120 days. For the survival experiments, long-term survivors (cures) were determined at 120 days post tumor implantation. Body weights were measured as a general measure of toxicity.

The subcutaneous tumor data were analyzed by determining the mean tumor volume for each treatment group over the course of the experiment and calculating the tumor growth delay as the difference in days for the treatment versus the control tumors to reach a volume of 500 and 1000 mm^3.

Treatment with the compound alone produced a tumor growth delay of 6 days at the higher dose administered in the murine Lewis lung carcinoma. Paclitaxel produced 4.5 days of tumor growth delay while the combination of paclitaxel and the compound resulted in 20 days of tumor growth delay. Carboplatin produced 5 days of tumor growth delay. Combining treatment with carboplatin and the compound resulted in 9 days of tumor growth delay. Gemcitabine produced 7 days of tumor growth delay while the combination of gemcitabine and the compound resulted in 16 days of tumor growth delay.

These increases reflect a greater-than-additive effect of the two treatments for paclitaxel and additive to greater-than-additive effects for carboplatin. These therapeutic effects were directly reflected in the response of the systemic disease as represented by lung metastases in animals bearing the Lewis lung carcinoma.

The human T98G glioblastoma multiforme was implanted subcutaneously in some animals and intracranially in other animals. Two treatment plans were studied: 1) simultaneous or overlapping treatment with the compound and cytotoxic therapy (BCNU); and 2) sequential treatment completing cytotoxic therapy (BCNU) first and then initiating treatment with the compound. Treatment with the compound (30 mg/kg) twice daily on days 4 through 18 post tumor implantation produced 9 days of T98G tumor growth delay. Administration of BCNU resulted in 4.5 days of tumor growth delay while the combination regimen produced 16 days of tumor growth delay. When animals with intracranial tumors were treated with the compound (30 mg/kg) on days 4 through 18 the mean survival was 72 days compared with 38 days survival in the control animals. Treatment of intracranial tumor-bearing animals with BCNU resulted in 42 days mean survival while combining treatment of BCNU with the compound produced a mean survival time of 102 days and 3 out of 5 of the animals were long-term survivors (120-day survivors or cures). These results demonstrate additive to greater-than additive tumor/therapeutic response to the combination treatment regimens.

For the sequential treatment regimen, administration of the compound salt occurred on days 12 through 30 post tumor implantation. In animals bearing subcutaneously implanted T98G tumors there was a dose dependent increase in tumor growth delay as the dose of the the compound was increased from 3 to 10 and then to 30 mg/kg with tumor growth delays of 16, 24 and 34 days, respectively. Treatment with BCNU produced a tumor growth delay of 4.5 days that when followed by treatment with the compound (30 mg/kg) increased to 47 days. Administration of the sequential treatment regimen to animals bearing T98G tumor implanted intracranially resulted in survival times up to 74 days with compound alone. Treatment with BCNU produced a survival time of 42 days that was increased to 78 days when followed by administration of the compound (30 mg/kg) on days 12 through 30, however, no long-term survivors were

obtained with the sequential treatment regimen. These results demonstrate additive to greater-than-additive tumor/therapeutic response to the combination treatment regimens.

The human Calu-6 non-small cell lung carcinoma (NSCLC) was grown as a subcutaneous xenograft in female nude mice. Animals bearing the Calu-6 non-small cell lung carcinoma xenograft were treated with combination regimens including the compound by the simultaneous and sequential treatment regimens. Treatment of animals bearing the Calu-6 tumor with the compound (30 mg/kg) twice daily on days 4 through 18 post tumor implantation resulted in 11 days of tumor growth delay. Paclitaxel, in a full murine dose regimen, produced 6 days of tumor growth delay in this tumor that increased to 23 days when combined with the compound. Administration of carboplatin to animals bearing the Calu-6 tumor produced 5 days of tumor growth delay that was increased to 15 days of tumor growth delay when combined with administration of the compound on days 4 through 18. For the sequential treatment regimen, the compound was administered on days 14 through 30 post Calu-6 tumor implantation and resulted in up to 9 days of tumor growth delay. Treatment with paclitaxel produced 6 days of tumor growth delay that increased to 23 days when followed by treatment with the compound (30 mg/kg). Treatment with carboplatin resulted in 5 days of tumor growth delay that increased to 21 days when carboplatin was followed by administration of the compound (30 mg/kg). These results demonstrate additive to greater-than-additive tumor response to the combination treatment regimens.

The human SW2 small cell lung carcinoma xenograft was grown subcutaneously in male nude mice and the compound was administered to the animals along with cytotoxic chemotherapy in the sequential treatment regimen. The tumor growth delay produced by administration of 30 mg/kg of the compound 317615.2HCl on days 14 through 30 to animals bearing the SW2 tumor was 10 days. Paclitaxel is a very effective antitumor agent against the SW2 tumor producing a tumor growth delay of 25 days. The combination treatment regimen with paclitaxel followed by the compound (30 mg/kg) resulted in a tumor growth delay of 62 days. Treatment with carboplatin produced a tumor growth delay of 5 days in the SW2 tumor that was increased to 14 days when carboplatin was followed by treatment with the compound (30 mg/kg). These results

demonstrate that these combination treatment regimens produce additive to greater-than-additive tumor response against the SW2 small cell lung carcinoma xenograft.

The human SKOV-3 ovarian carcinoma was implanted intraperitoneally in female nude mice and the endpoint for the experiment was increase-in-lifespan (survival) of the animals. There was a dose dependent increase in the survival of animals treated with the compound with a maximal mean survival time of 72 days with a dose of the compound of 30 mg/kg while the control animals had a mean survival time of 39 days. Paclitaxel is a very effective antitumor agent in ovarian carcinoma and produced a survival time in animals bearing the SKOV-3 tumor of 84 days. The sequential combination treatment regimen of paclitaxel followed by the compound at compound doses of 10 or 30 mg/kg resulted in 5 out of 5 animals in these groups being long-term survivors or cures when the experiment was terminated at 120 days post tumor implantation. Treatment with carboplatin resulted in a mean survival time of 45 days in animals bearing the SKOV-3 tumor and that was increased to 80 days when carboplatin was followed by administration of the compound on days 14 through 30 post tumor implantation.

The human HT-29 colon carcinoma xenograft was grown subcutaneously in female nude mice. 5-Fluorouracil is the most widely used anticancer agent for the treatment of colon carcinoma. 5-Fluorouracil or cisplatin was combined with treatment with the compound in the simultaneous combination treatment regimen in animals bearing HT-29 colon carcinoma xenografts. Treatment with the compound (30 mg/kg) on days 4 through 25 post tumor implantation produced a 15 day tumor growth delay in the HT-29 colon carcinoma. Administration of 5-fluorouracil on days 7 through 11 produced a tumor growth delay of 7.5 days while combination treatment with the compound (30 mg/kg) along with 5-fluorouracil resulted in 17 days of tumor growth delay. Treatment with cisplatin produced a tumor growth delay of 6 days in the HT-29 colon carcinoma that increased to 20 days when combined with administration of the compound (30 mg/kg). These results demonstrate additive to greater-than-additive tumor response to the combination treatment regimens.

The human MX-1 breast carcinoma xenograft was grown subcutaneously in female nude mice. When animals bearing the MX-1 tumor were treated with the compound (30 mg/kg) on days 4 through 25 post tumor implantation and a tumor growth

delay of 20 days resulted . Paclitaxel was a very effective antitumor agent in the MX-1 tumor producing a tumor growth delay of 37 days that was increased to 62 days when administered in simultaneous combination with the compound (30 mg/kg). Treatment with carboplatin resulted in a tumor growth delay of 10 days that was increased to 18 days when administered in simultaneous combination with the compound. These results demonstrate additive to greater-than-additive tumor response for the combination treatment regimens.

Both the simultaneous and overlapping treatment regimen and the sequential treatment regimen were used to assess the compound alone and along with 5-fluorouracil or gemcitabine against the human HS746T gastric carcinoma xenograft. The compound was effective in this tumor model. Administration of the compound alone on days 4 through 18 post tumor implantation over a dosage range from 3 to 30 mg/kg produced tumor growth delays between 6.5 and 15 days while administration of the compound alone on days 14 through 30 post tumor implant over the same dosage range resulted in tumor growth delays of 0.8 to 19 days in the HS746T gastric carcinoma. The HS746T had a limited response to 5-fluorouracil and treatment with that drug alone produced a 3 day tumor growth delay of the HS746T tumor. Treatment with 5-fluororuacil simultaneously along with the compound (30 mg/kg) resulted in 15 days of tumor growth delay. Treatment with 5-fluorouracil followed by the compound (30 mg/kg) resulted in 8.5 days of tumor growth delay. The HS746T gastric carcinoma was more responsive to gemcitabine resulting in a tumor growth delay of 15 days in this tumor. Simultaneous treatment with gemcitabine along with the compound (30 mg/kg) produced a tumor growth delay of 40 days. Sequential treatment with gemcitabine followed by the compound resulted in 30 days of tumor growth delay.

Both the simultaneous and overlapping treatment regimen and the sequential treatment regimen were used to assess the compound alone and along with 5-fluorouracil or gemcitabine against the human Hep3B hepatocellular carcinoma xenograft. The compound was effective in this tumor model. Administration of the compound alone on days 4 through 18 post tumor implantation over a dosage range from 3 to 30 mg/kg produced tumor growth delays between 5 and 25 days while administration of the compound alone on days 14 through 30 post tumor implant over the same dosage range

resulted in tumor growth delays of 4 to 22 days in the Hep3B hepatocellular carcinoma. The Hep3B had a moderate response to 5-fluorouracil and treatment with that drug alone produced a 9 day tumor growth delay of the Hep3B tumor. Treatment with 5-fluororuacil simultaneously along with the compound (30 mg/kg) resulted in 31 days of tumor growth delay. Treatment with 5-fluorouracil followed by the compound (30 mg/kg) resulted in 43 days of tumor growth delay. The Hep3B hepatocellular carcinoma was also moderately responsive to gemcitabine resulting in a tumor growth delay of 11 days in this tumor. Simultaneous treatment with gemcitabine along with the compound (30 mg/kg) produced a tumor growth delay of 28 days. Sequential treatment with gemcitabine followed by the compound resulted in 35 days of tumor growth delay.

Both the simultaneous and overlapping treatment regimen and the sequential treatment regimen were used to assess the compound alone and along with fractionated radiation therapy or gemcitabine against the human CaKi1 renal cell carcinoma xenograft. The compound was effective in this tumor model. Administration of the compound alone on days 4 through 18 post tumor implantation over a dosage range from 3 to 30 mg/kg produced tumor growth delays between 10 and 15 days while administration of the compound alone on days 14 through 30 post tumor implant over the same dosage range resulted in tumor growth delays of 4 to 14 days in the CaKi1 renal cell carcinoma. The CaKi1 renal cell carcinoma was quite responsive to gemcitabine resulting in a tumor growth delay of 19.5 days in this tumor. Simultaneous treatment with gemcitabine along with the compound (30 mg/kg) produced a tumor growth delay of 29 days. Sequential treatment with gemcitabine followed by the compound resulted in 24 days of tumor growth delay.

The murine Lewis lung carcinoma or human colon HT-29 colon carcinoma, CaKi1 renal cell carcinoma or human MX-1 breast carcinoma was implanted in male C57Bl mice or female nude mice and the tumor-bearing animals were treated with the compound alone or along with fractionated radiation therapy (2, 3 or 4 Gray fractions) delivered using a GammaCell 40 cesium irradiator. Specifically, tumor cells prepared from a brie of donor tumors (5×10^6 cells) were implanted subcutaneously in a hind-leg of mice (Charles River). Treatment with the compound (10 or 30 mg/kg) administered orally twice per day for schedules lasting 2 weeks or more. Each treatment group as well

as a group of untreated control animals consisted of 5 animals per group in each experiment. Subcutaneous tumor response was monitored by tumor volume measurement performed twice per week over the course of 60-120 days. Body weights were measured as a general measure of toxicity.

The subcutaneous tumor data were analyzed by determining the mean tumor volume for each treatment group over the course of the experiment and calculating the tumor growth delay as the difference in days for the treatment versus the control tumors to reach a volume of 500 and 1000 mm^3.

Treatment with the compound alone produced a tumor growth delay of 2.5 days at the higher dose administered in the murine Lewis lung carcinoma. Fractionated radiation therapy produced increasing tumor growth delay with increasing radiation dose reaching a maximum tumor growth delay of 9 days with a regimen of 5 x 4 Gray. Administration of the compound along with fractionated radiation therapy had a very marked effect on tumor response especially at the lower radiation doses of 2 and 3 Gray. The parallel nature of the dose response curves implies that treatment with the compound allows the radiation delivered to kill a population of tumor cells not effected by radiation alone.

The human HT-29 colon carcinoma xenograft was grown subcutaneously in female nude mice and was treated for a two week regimen with fractionated radiation therapy (2, 3 or 4 Gray fractions). Radiation therapy alone produced increasing tumor growth delay with increasing dose of radiation reaching a maximum growth delay of 24 days. Administration of the compound (30 mg/kg) along with fractionated radiation therapy resulted in increased tumor growth delay at each radiation dose reaching 31 days at the highest radiation dose tested.

The CaKi1 tumor was quite sensitive to fractionated radiation therapy (3 Gray x 10) and treatment with that modality alone produced an 18.5 day tumor growth delay of the CaKi1 tumor. Treatment with fractionated radiation therapy simultaneously along with the compound (30 mg/kg) resulted in 25 days of tumor growth delay. Treatment with fractionated radiation therapy followed by the compound (30 mg/kg) resulted in 32.5 days of tumor growth delay.

The human MX-1 breast carcinoma xenograft was grown subcutaneously in female nude mice and was treated for a two week regimen with fractionated radiation

therapy (2, 3 or 4 Gray fractions). Radiation therapy alone produced increasing tumor growth delay with increasing doses of radiation reaching a maximum growth delay of 84 days. Administration of the compound (30 mg/kg) along with fractionated radiation therapy resulted in increased tumor growth delay at each radiation dose reaching 100 days at the highest radiation dose tested.

The PKC inhibitor of the present invention is administered in combination with other anti-neoplasm therapies including anti-neoplastic agents and radiation therapy. The phrase "in combination with" means that the compound of Formula I can be administered shortly before, shortly after, concurrently, or any combination of before, after, or concurrently, with such other anti-neoplasm therapies. Thus, the compound and the anti-neoplastic agent can be administered simultaneously as either as a single composition or as two separate compositions or sequentially as two separate compositions. Likewise, the compound and radiation therapy can be administered simultaneously, separately or sequentially. The compound can be administered in combination with more than one anti-neoplasm therapy. In a preferred embodiment, the compound is administered from 2 weeks to 1 day before any chemotherapy, or 2 weeks to 1 day before any radiation therapy. In another preferred embodiment, the PKC inhibitor can be administered during anti-neoplastic chemotherapies and radiation therapies. If administered following such chemotherapy or radiation therapy, the PKC inhibitor should be given within 1 to 14 days following the primary treatments. The PKC inhibitor may also be administered chronically or semi-chronically, over a period of from about 2 weeks to about 5 years

One skilled in the art will recognize that the amount of PKC inhibitor to be administered in accordance with the present invention in combination with other anti-neoplastic agents or therapies is that amount sufficient to enhance the anti- neoplasm effects of anti-neoplastic agents or radiation therapies or that amount sufficient to induce apoptosis or cell death along with the anti-neoplastic or radiation therapy and/or to maintain an antiangiogenic effect. Such amount may vary, among other factors, depending upon the size and the type of neoplasia, the concentration of the compound in the therapeutic formulation, the specific anti-neoplasm agents used, the timing of the

administration of the PKC inhibitors relative to the other therapies, and the age, size and condition of the patient.

Generally, an amount of protein kinase C inhibitor to be administered in combination with other anti-neoplasm therapies is decided on a case by case basis by the attending physician. As a guideline, the extent of the neoplasia, the body weight, and the age of the patient will be considered, among other factors, when setting an appropriate dose.

Generally, a suitable dose is one that results in a concentration of the protein kinase C inhibitor at the site of tumor cells in the range of 50 nM to 50 μM, and more usually from 100 nM to 10 μM. It is expected that serum concentrations of 250 nM to 250 μM should be sufficient in most circumstances.

To obtain these treatment concentrations, a patient in need of treatment will be administered between about 0.5 mg per day per kg of body weight and about 10 mg per day per kg. Usually, not more than about 3 mg per day per kg of body weight of protein kinase C inhibitor should be needed. As noted above, the above amounts may vary on a case-by-case basis.

The compound of the present invention is preferably formulated prior to administration. Suitable pharmaceutical formulations are prepared by known procedures using well known and readily available ingredients. In making the compositions suitable for use in the method of the present invention, the active ingredient will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semisolid or liquid material which acts as a vehicle, excipient or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosol (as a solid or in a liquid medium), soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders for either oral or topical application.

Some examples of suitable carriers, excipient, and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphates, alginate, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone,

cellulose, water syrup, methyl cellulose, methyl and propylhydroxybenzoates, talc, magnesium stearate and mineral oil. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions of the invention may be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient. The compositions are preferably formulated in a unit dosage form, each dosage containing from about 0.05 mg to about 3 g, more usually about 64 mg of the active ingredient. However, it will be understood that the therapeutic dosage administered will be determined by the physician in the light of the relevant circumstances including the severity of the condition to be treated, the choice of compound to be administered and the chosen route of administration. Therefore, the above dosage ranges are not intended to limit the scope of the invention in any way. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier.

In addition to the above formulations, most of which may be administered orally, the compound used in the method of the present invention also may be administered topically. Topical formulations include ointments, creams and gels.

Ointments generally are prepared using either (1) an oleaginous base, i.e., one consisting of fixed oils or hydrocarbons, such as white petrolatum or mineral oil, or (2) an absorbent base, i.e., one consisting of an anhydrous substance or substances which can absorb water, for example anhydrous lanolin. Customarily, following formation of the base, whether oleaginous or absorbent, the active ingredient (compound) is added to an amount affording the desired concentration.

Creams are oil/water emulsions. They consist of an oil phase (internal phase), comprising typically fixed oils, hydrocarbons, and the like, such as waxes, petrolatum, mineral oil, and the like, and an aqueous phase (continuous phase), comprising water and any water-soluble substances, such as added salts. The two phases are stabilized by use of an emulsifying agent, for example, a surface active agent, such as sodium lauryl sulfate; hydrophilic colloids, such as acacia colloidal clays, veegum, and the like. Upon

formation of the emulsion, the active ingredient (compound) customarily is added in an amount to achieve the desired concentration.

Gels comprise a base selected from an oleaginous base, water, or an emulsion-suspension base. To the base is added a gelling agent which forms a matrix in the base, increasing its viscosity. Examples of gelling agents are hydroxypropyl cellulose, acrylic acid polymers, and the like. Customarily, the active ingredient (compounds) is added to the formulation at the desired concentration at a point preceding addition of the gelling agent.

The amount of compound incorporated into a topical formulation is not critical; the concentration should be within a range sufficient to permit ready application of the formulation to the affected tissue area in an amount which will deliver the desired amount of compound to the desired treatment site.

The customary amount of a topical formulation to be applied to an affected tissue will depend upon an affected tissue size and concentration of compound in the formulation. Generally, the formulation will be applied to the effected tissue in an amount affording from about 1 to about 500 μg compound per cm^2 of an affected tissue. Preferably, the applied amount of compound will range from about 30 to about 300 $\mu g/cm^2$, more preferably, from about 50 to about 200 $\mu g/cm^2$, and, most preferably, from about 60 to about 100 $\mu g/cm^2$.

The following formulation examples are illustrative only and are not intended to limit the scope of the invention in any way.

Formulation 1

Hard gelatin capsules are prepared using the following ingredients:

	Quantity (mg/capsule)
Active agent	250
starch, dried	200
magnesium stearate	10
Total	460 mg

The above ingredients are mixed and filled into hard gelatin capsules in 460 mg quantities.

Formulation 2

A tablet is prepared using the ingredients below:

	Quantity (mg/capsule)
Active agent	250
cellulose, microcrystalline	400
silicon dioxide, fumed	10
stearic acid	5
Total	665 mg

The components are blended and compressed to form tablets each weighing 665 mg.

Formulation 3

Tablets each containing 60 mg of active ingredient are made as follows:

	Quantity (mg/tablet)
Active agent	60 mg
starch	45 mg
microcrystalline cellulose	35 mg
polyvinylpyrrolidone (as 10% solution in water)	4 mg
sodium carboxymethyl starch	4.5 mg
magnesium stearate	0.5 mg
talc	1 mg
Total	150 mg

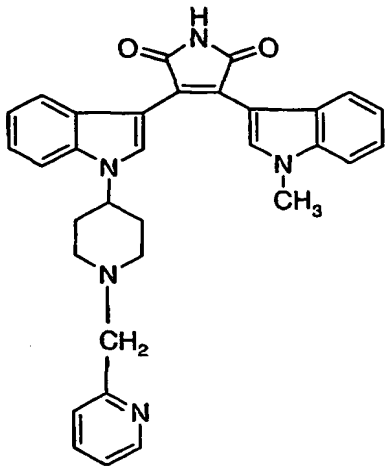
The active ingredient, starch and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°C and passed through a No. 18 mesh U.S. sieve.

The sodium carboxymethyl starch, magnesium stearate and talc, previously passed through a No. 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

The principles, preferred embodiments and modes of operation of the present invention have been described in the foregoing specification. The invention which is intended to be protected herein, however, is not to be construed as limited to the particular forms disclosed, since they are to be regarded as illustrative rather than restrictive. Variations and changes may be made by those skilled in the art without departing from the spirit of the invention.

CLAIMS:

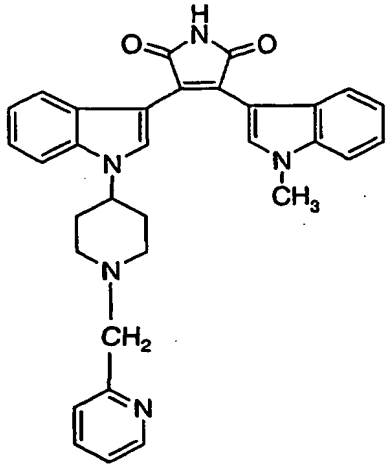
1. A method for treating a neoplasm which comprises administering to a mammal in need thereof, an anti-neoplastic agent in combination with a compound of the formula



or a pharmaceutically acceptable salt or solvate thereof.

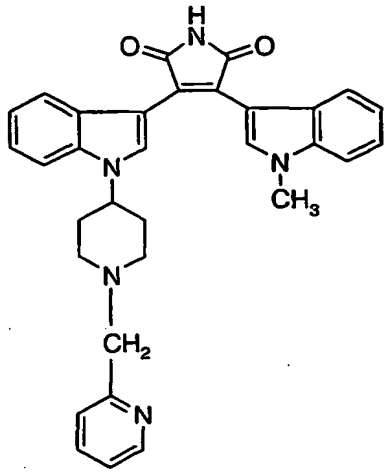
2. The method of claim 1, wherein the anti-neoplastic agent is selected from the group consisting of Ara-c, VP-16, *cis*-platin, adriamycin, 2-chloro-2- deoxyadenosine, 9-β-D-arabinosyl-2-fluoroadenine, carboplatin, gemcitabine, paclitaxel, BCNU, 5-fluorouracil, and doxorubicin.

3. A method for treating a neoplasm which comprises administering to a mammal in need thereof, therapeutic radiation having an anti-neoplastic effect in combination with a compound of the formula:



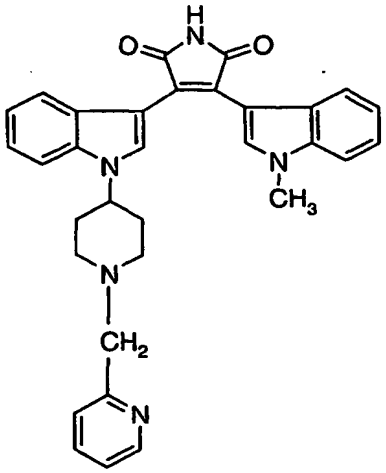
or a pharmaceutically acceptable salt or solvate thereof.

4. A method for enhancing the anti-neoplastic effect of an anti-neoplastic agent in a mammal which comprises administering to a mammal in need thereof, a compound of the formula:



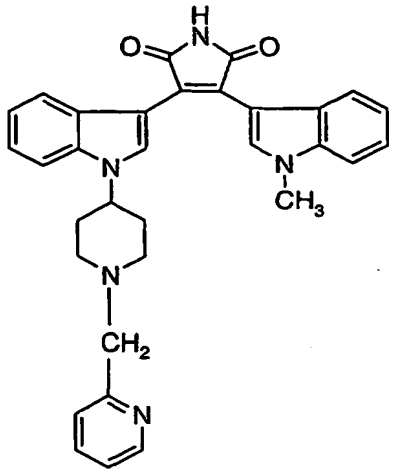
or a pharmaceutically acceptable salt or solvate thereof, in combination with an anti-neoplastic agent.

5. A method for enhancing the anti-neoplastic effect of therapeutic radiation in a mammal which comprises administering to a mammal in need thereof, a compound of the formula:



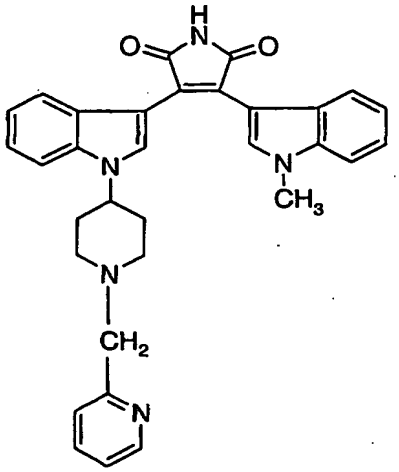
or a pharmaceutically acceptable salt or solvate thereof, in combination with therapeutic radiation having an anti-neoplastic effect.

6. The use of a compound of the formula:



or a pharmaceutically acceptable salt or solvate thereof, for the manufacture of a medicament for enhancing the treatment of a neoplasm.

7. The use of a compound of the formula:



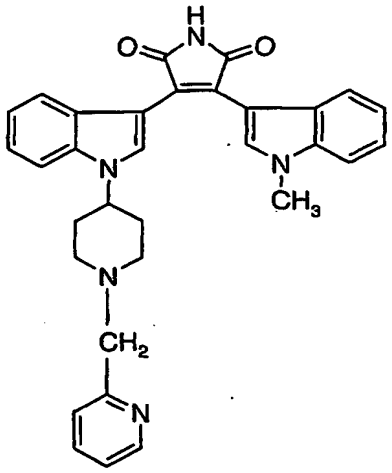
or a pharmaceutically acceptable salt or solvate thereof, for the manufacture of a medicament for the treatment of a neoplasm which treatment comprises administration of the said compound in combination with an anti-neoplastic agent.

8. The use according to claim 7 wherein the treatment comprises the simultaneous, separate or sequential administration of the said compound with the anti-neoplastic agent.

9. The use according to claim 7 or 8 wherein the anti-neoplastic agent is selected from the group consisting of alkylating agents, antibiotics and plant alkaloids, hormones and steroids, synthetic agents having anti-neoplastic activity, antimetabolites and biological molecules having anti-neoplastic activity.

10. The use according to any one of claims 7 to 9 wherein the anti-neoplastic agent is selected from the group consisting of Ara-c, VP-16, *cis*-platin, adriamycin, 2-chloro-2- deoxyadenosine, 9-β-D-arabinosyl-2-fluoroadenine, carboplatin, gemcitabine, paclitaxel, BCNU, 5-fluorouracil, and doxorubicin.

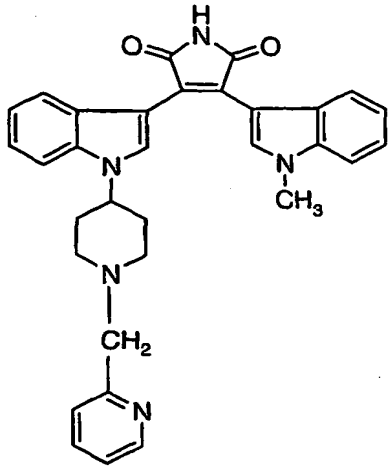
11. The use of a compound of the formula:



or a pharmaceutically acceptable salt or solvate thereof, for the manufacture of a medicament for the treatment of a neoplasm which treatment comprises the administration of the said compound in combination with therapeutic radiation having an anti-neoplastic effect.

12. The use according to claim 11 wherein the treatment comprises the simultaneous, separate or sequential administration of the said compound with the said radiation.

13. A product containing a compound of formula:



or a pharmaceutically acceptable salt or solvate thereof and an anti-neoplastic agent as a combined preparation for the simultaneous, separate or sequential use in treating a neoplasm.

14. A product according to claim 13 wherein the anti-neoplastic agent is selected from the group consisting of alkylating agents, antibiotics and plant alkaloids, hormones and steroids, synthetic agents having anti-neoplastic activity, antimetabolites and biological molecules having anti-neoplastic activity.

15. A product according to claim 13 or 14 wherein the anti-neoplastic agent is selected from the group consisting of Ara-c, VP-16, *cis*-platin, adriamycin, 2-chloro-2-deoxyadenosine, 9-β-D-arabinosyl-2-fluoroadenine, carboplatin, gemcitabine, paclitaxel, BCNU, 5-fluorouracil, and doxorubicin.

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(54) Title: USE OF A PROTEIN KINASE C INHIBITOR TO ENHANCE THE CLINICAL EFFICACY OF ANTI-NEOPLASTIC CHEMOTHERAPEUTIC AGENTS AND RADIATION THERAPY

(57) Abstract: Methods are disclosed for treating a neoplasm which comprises administering to a mammal in need thereof, an anti-neoplastic agent or therapeutic radiation in combination with 3-[1-(1-(pyridin-2-ylmethyl)piperidin-4-yl)-indol-3-yl]-4-(1-methylindol-3-yl)-1H-pyrrole-2,5-dione or a pharmaceutically acceptable salt or solvate thereof, and for enhancing the anti-neoplastic effect of anti-neoplasticagents or therapeutic radiation which comprises administering to a mammal in need thereof, 3-[1-(1-(pyridin-2-ylmethyl)piperidin-4-yl)-indol-3-yl]-4-(1-methylindol-3-yl)-1H-pyrrole-2,5-dione in combination with said anti-neoplastic agent or said therapeuticradiation having an anti-neoplastic effect.